PHYTOPATHOLOGISCH LABORATORIUM

„WILLIE COMMELIN SCHOLTEN"

BAARN

MEDEDELING No. 33

THE PARASITISM OF EXOBASIDIUM JAPONICUM SHIR. ON AZALEA

DOOR

W. GRAAFLAND

Done

REPRINT FROM: ACTA BOTANICA NEERLANDICA 9 (4), 1960

COMMONWEALTH
21 FEB 1961

Acta Botanica Neerlandica **9** (1960) 347–379

THE PARASITISM OF EXOBASIDIUM JAPONICUM SHIR. ON AZALEA

W. GRAAFLAND

(*Phytopathological Laboratory "Willie Commelin Scholten", Baarn*)

(*received August 4th, 1960*)

CONTENTS

		page
CHAPTER I.	INTRODUCTION	347
CHAPTER II.	TAXONOMY AND CULTIVATION OF AZALEAS	349
CHAPTER III.	OCCURRENCE OF THE DISEASE	351
CHAPTER IV.	THE FUNGUS	352
	4.1. Review of literature	352
	4.2. Description of the fungus on Azalea	353
	4.3. Anatomy of the galls on Azalea	354
CHAPTER V.	THE MATERIAL USED FOR THE EXPERIMENTS	355
	5.1. The plant material	355
	5.2. The isolation and cultivation of *Exobasidium japonicum*	355
CHAPTER VI.	INOCULATION EXPERIMENTS	357
	6.1. Literature	357
	6.2. Experiments	358
CHAPTER VII.	LIFE CYCLE OF *Exobasidium* SPECIES	359
	7.1. Literature	359
	7.2. Experiments	360
CHAPTER VIII.	CROSS INOCULATIONS	363
	8.1. Cross inoculations with *Exobasidium* spores originating from different Azalea cultivars	363
	8.2. Cross inoculations with *Exobasidium* spores originating from different less closely related host plants	364
CHAPTER IX.	DEVELOPMENTAL STAGES OF THE BUD AT WHICH INFECTION WITH *Exobasidium japonicum* IS POSSIBLE	366
CHAPTER X.	CONTROL OF THE DISEASE	369
	10.1. Hygienic measures	369
	10.2. Control by fungicides	369
	10.3. Experiments with fungicides in vitro	370
	10.4. Experiments with fungicides in vivo	371
CHAPTER XI.	DISCUSSION OF THE RELATIONS BETWEEN THE GENERA *Exobasidium, Ustilago, Tilletia* AND *Taphrina*	374
SUMMARY		376
REFERENCES		378

CHAPTER I

INTRODUCTION

The growing of Azaleas plays an important part in many horticultural centres of Western Europe, such as Dresden in Germany, Ghent in Belgium, and Boskoop in the Netherlands. Every year

Dutch growers spend large sums for buying Azaleas, mostly young plants, from the Belgian horticulturists. In the Dutch nurseries these plants are grown until they begin to flower, and are ready to be sold. The large assortment of Azaleas that is grown under the names *Azalea indica* and *Azalea obtusa*, consists of a great variety of cultivars which, botanically speaking, all belong to the *Rhododendron* group.

Often these plants suffer from a disease known in Dutch as "oortjes ziekte" or "ezelsoren", indicating that the deformed leaves are shaped like ears. The diseased leaves are greatly enlarged and thicker than normal, the leafblades becoming convex and crooked because of irregular growth. The original shape of the leaves can no longer be recognized, and it is understandable that these misshapes reminded people of ears (Plate 1, A and B). In this article these hypertrophic organs will be called galls.

The disease is caused by a fungus known as *Exobasidium japonicum* Shir. and belonging to the *Basidiomycetes*. The mycelium of the fungus present in the intercellular spaces of the leaf tissue, produces the hymenium, which develops under the epidermis, and consists of a layer of cells, the basidia. After breaking through the epidermal cells, the basidia form basidiospores, and as the latter are produced in enormous numbers, they give the galls a velvety appearance. The colour of the diseased parts becomes white to reddish at that stage. The galls develop gradually, often unobserved, as they are frequently hidden under flowers and leaves. When the flowering period is over, they seem to appear suddenly, to the surprise of the owner of the Azalea, who thought himself in possession of a healthy plant. This disease was observed for the first time in Western Europe at the turn of this century. It is possible that it was imported with plants from Japan, for the fungus was already described in that country by SHIRAI (1896).

The lifecycle of *Exobasidium* on Azalea is only incompletely known. Thus, the first purpose of the experiments described in this paper, was to obtain better information concerning the nature of the parasitism of this fungus. An answer to the following questions was still lacking: at what stage of the development of the plant does the parasite penetrate and under which circumstances; is one basidiospore sufficient to produce an infection, or is the fungus heterothallic?

Another question to be answered was how the length of the incubation period varies under different conditions. The presence of the symptoms can easily be observed, but the moment of infection is as yet unknown. Therefore, it is difficult for the growers to know when preventive measures have to be carried out. Some experiments were carried out with a small number of fungicides, which were applied before and after the Azaleas were inoculated with the parasite. This kind of research, however, will have to be continued in large-scale cultures.

In this paper some attention is also paid to the nomenclature of the Azalea parasite. SHIRAI (1896) has been the first to describe an *Exobasidium* on the 'Indian Azalea'. American mycologists described

parasites on wild *Rhododendron* species and on other members of the *Ericaceae* family occurring in North America as new species of the genus *Exobasidium*. It is still an open question whether all these species are distinct, or whether the parasites described by Shirai and the American authors are identical with *Exobasidium vaccinii* named and described by Woronin in 1867. If the various species should prove to be conspecific, this name has priority. Many authors are of opinion that they actually are conspecific, as no morphological distinction can be found between the parasites living on different host plants. The results of cross inoculations with *Exobasidium* spores of different origin would enable us to answer the question whether these parasites are fully identical, or whether they show specialization on their host plants. To that purpose a collection of host plants with their parasites is indispensable. The experiments described in this paper were, however, restricted to cross inoculations with *Exobasidium* originating from Azalea and from *Vaccinium vitis idaea*. This *Vaccinium* and some Azalea cultivars were used as host plants.

The purpose of this investigation was to obtain an answer to the aforesaid questions concerning the life cycle of the *Exobasidium* parasite on Azalea, and to investigate whether this fungus is identical with that described by Woronin. Finally, the striking similarity between the parasitism of *Exobasidium* spp. and that shown by *Taphrina* spp., belonging to the *Ascomycetes*, will be discussed.

CHAPTER II

TAXONOMY AND CULTIVATION OF AZALEAS

Linnaeus described in his Species Plantarum (1753) the red Alpine Rose, which he named *Rhododendron ferrugineum*. This, therefore, is the type of the genus *Rhododendron*.

About a century later Hooker's expedition to the Himalayas brought 45 new *Rhododendron* species to Western Europe, which were described in 1851. More new species were introduced into Europe from America as well as from Western China, Japan, Burma, Indo-China, Malaya and other Asiatic countries. At present about 800 to 900 species are known, including hardy ones, called *Rhododendron* and non-hardy ones, which shed their leaves under unfavourable conditions. The latter were placed in the genus *Azalea*. However, as hybridization between more and less hardy species is easy, it is not justified to retain *Azalea* as a distinct genus. It should be sunk into *Rhododendron*. The latter can be divided into two subgenera: *Eurhododendron* and *Anthodendron*. To the former are assigned the *Rhododendron* species bearing leathery leaves which are not shed during winter. In the subgenus *Anthodendron* are placed about 70 non-hardy, soft-leaved species, which in practice are called Azalea. As the name Azalea is generally used by the growers, the name will be used in this paper too. All experiments were performed with *Rhododendron* plants of this type. The Japanese Azaleas may be assigned to the 'Tsutsutsi' section

of the subgenus *Anthodendron*. They are also called 'Kurume' Azaleas. *Rhododendron obtusum* Planch. was obtained by a crossing of the hardy *Rhododendron kaempferi*, originating from the Japanese plains, with other Japanese species. The grower W. F. Koppeschaar obtained the small- and pink-flowered cultivar 'Esmeralda' from Japanese seed. Other cultivars grown at the Boskoop centre of horticulture are 'Galestin' and 'Moederkensdag'. They all belong to the 'Kurume' group, and are designated by the growers as *Azalea obtusa*. The Indian Azalea has *Rhododendron simsii* Planch. as ancestor, and is called by the growers *Azalea indica*. A hybrid of this species and a *Rhododendron obtusum* var. *amoenum* is the cultivar 'Hexe' frequently grown in Belgian nurseries (GROOTENDORST, 1954; ENCKE, 1959).

For the cultivation of Azaleas soil is used from the oak and fir woods of the "Campine", a region in the northern part of Belgium. The plants are grown from seed or from cuttings. When 8 months old, the "baby Azaleas" are in possession of 6 to 8 leaves, and at the age of 18 months they can be sold. The cuttings are obtained by trimming the plants either in August to September, or during the period between February and April. The humidity of the air in the rooting beds has to be high. Young plants, i.e. plants which have to be kept in the nursery for a second year before they start flowering, are frequently bought by Dutch growers from Belgian firms. The value of the *Azalea indica* imports from Belgium and Luxembourg into the Netherlands in 1957 was estimated at about 1.2 million Dutch guilders (VANDENDAEL, 1960). In that year, according to the "Tuinbouwgids", 1960 (Dutch Horticultural Guide), 540.000 *Azalea indica* and *Azalea obtusa* plants were sold at the Dutch auctions.

The plants are not hardy under our climatological conditions. Most varieties react on an exposure to night frost by shedding their leaves. Thus they have to be kept in the greenhouses till the beginning of May, when they are brought into the open (VAN RAALTE, 1955). However, protection against late night frosts is still necessary. They are densely planted in the beds. In autumn they have to be returned to the greenhouse. During autumn and winter they are exposed to a temperature of about 16° C, which may be raised gradually at the time of the development of the flower buds. However, if the temperature becomes too high, which may occur in early spring, the doors of the greenhouses are opened in order to lower the temperature. In all cases a high humidity of the air and a high moisture content of the soil are conditions for good development. Therefore, the plants are continuously sprayed, often by means of mist sprayers, during the time the plants are outside as well as during the time they are kept in the glasshouses. This spraying and the opening of the glasshouse doors in order to lower the temperature are the cause of heavy air-currents blowing through the glasshouses. In this way micro-organisms, including spores of pathogens such as *Exobasidium japonicum*, can be spread easily from diseased to healthy plants. The high humidity of the air keeps the spores viable and favours infection.

CHAPTER III

OCCURRENCE OF THE DISEASE

In the centra of the Azalea cultivation the *Exobasidium* disease, already known for over half a century still occurs frequently. Susceptible varieties, such as 'Hexe', may be diseased to such an extent that the same plant may seem to carry besides its normal red flowers also somewhat deformed white ones. The latter are the many big galls covered with enormous amounts of spores. Though the galls appear throughout the year, gall formation has an optimum in certain months. An inquiry among the Belgian growers revealed that galls are most frequent during March and April; at this time the flowering period is finished, but the plants are still kept in the greenhouses. During June galls may be frequent on plants in the open, and sometimes this may be so in September too (Welvaert, 1952). One gets the impression that most of the galls appear at the time the plants are resuming their vegetative development. This occurs after flowering, when underneath the whithering flowers the axillary buds begin to develop. Renewed vegetative growth also occurs some weeks after the plants have been moved to the outside, i.e. in June. It has become clear from these facts as well as from our own observations that galls may appear as the buds start to unfold. The young galls become visible as small, somewhat swollen, translucent spots on the young leaves. By hypertrophic growth they soon become more conspicuous. They turn into galls of an irregular shape, and with a light green colour and a smooth, shining surface. When the fungus starts sporulating, the basidia rupture the cuticle, and the basidiospores are formed. At that time the surface of the galls gets a powdery aspect, and the colour changes from light green to snow-white. On account of the development of secundary spores sprouting from the basidiospores, the surface of the galls subsequently becomes covered with clusters of these spores, for which, as will be expounded here after, the name blastospores will be used; this gives them a velvety aspect. The diseased tissues then rapidly disintegrate: the galls shrivel, become brown and soon fall prey to all sorts of saprophytes. The parasite may attack all parts of the plant except root tissue. Stems and leaves as well as flower parts, therefore, may show hypertrophic growth caused by the parasite. Most galls, however, occur on the leaves, which usually are only partially attacked. The hypertrophic part is sharply separated from the healthy leaf tissue.

From these observations it was assumed that a bud can be infected when still in a dormant state. This hypothesis is in agreement with the observation that young galls are never present on fully developed leaves. The galls always appear simultaneously with the expanding of the young but still folded leaves of a developing bud. The resumption of growth by the dormant bud would coincide with a renewal of the activity of the pathogen. This would also explain the sudden appearance of the galls in plants which were apparently healthy

when imported. This mode of development would be in agreement with that described by WOLF and WOLF (1952) for *Exobasidium camelliae* var. *gracilis* Shirai on *Camellia sasanqua*.

CHAPTER IV

THE FUNGUS

4.1. REVIEW OF LITERATURE

The genus *Exobasidium* was proposed by WORONIN (1867), when he described the fungus causing a disease in *Vaccinium vitis idaea* as *Exobasidium vaccinii*. Though FUCKEL (1861) had observed the fungus already at an earlier date, this author did not give a good description of the pathogen, which he mistook for a *Fusidium*. After the appearance of Woronin's publication, many other *Exobasidium* species were described. Usually they were named after the host plants on which they occurred, but knowledge of their pathogenicity was lacking. The first *Exobasidium* occurring on a species of the genus *Rhododendron* was described by ELLIS (1874) as *Exobasidium discoideum*. It occurred on a North American wild species, viz. *Azalea viscosa*. Its basidiospores have a length of 20 μ. In the same year PECK (1874) described *Exobasidium azaleae* from the wild *Azalea nudiflora* growing in the same country. In this species the length of the basidiospores is 15–20 μ. Both authors paid much attention to the shape of the galls. SHIRAI (1896) described a parasite occurring on *Azalea indica* in Japan as *Exobasidium japonicum*. The length of the basidiospores of this parasite was 14–15 μ. RITZEMA BOS (1901) was the first to report an attack by *Exobasidium azaleae* Peck observed in a Dutch nursery in 1900. In a Belgian nursery an attack of *Azalea indica* was mentioned by LAUBERT (1909). This *Exobasidium*, which probably had been imported from Japan, would be identical either with *Exobasidium japonicum* Shir. or with *Exobasidium pentasporium* Shir. In Germany the disease was discovered in 1906 (NAUMANN, 1909). RACIBORSKI (1909) determined a parasite on *Rhododendron flavum* as *Exobasidium discoideum* Ellis. RICHARDS (1896) was already in doubt about the validity of many of the *Exobasidium* species created on account of their presence on different hosts, and often called after the latter. He could prove that *Exobasidium andromedae* Peck was identical with *Exobasidium vaccinii* Wor., as both parasites were able to cause similar disease symptoms on *Andromeda ligustrina*. In morphological respect several species prove to be indistinguishable. FARLOW (1877) considers *Exobasidium discoideum* Ellis and *Exobasidium azaleae* Peck as identical with *Exobasidium vaccinii* Wor., as no morphological differences between these species could be found. According to this author the names of the first mentioned species are superfluous, and only that of the last one should be retained.

NAUMANN (1909) was cautious in his conclusion concerning the identity of the Exobasidia occurring in Germany. He considers it to be impossible to identify the parasites on the base of their morpho-

logy alone. Many authors, however, agree with Farlow that the differences in size between the basidiospores of *Exobasidium vaccinii* Wor. and of several of the *Exobasidium* species that were described afterwards are so small that these parasites have to be considered as belonging to one species; the latter would comprise all *Exobasidium* species occurring on *Rhododendron* species (Lind, 1913; Burt, 1915; Laubert, 1925; Miles, 1928; and Savile, 1959). Marchionatto (1929) too considers *Exobasidium vaccinii* Wor. the cause of galls occurring on Azaleas cultivated in Argentina. According to this author, *Exobasidium azaleae* Peck, *Exobasidium discoideum* Ellis and *Exobasidium rhododendri* Cramer are identical with *Exobasidium vaccinii* Wor. Laubert (1909 and 1932) retains for the parasite on Azalea the name *Exobasidium japonicum* Shir., though he remarks that the difference between this species and that described by Woronin is not clear. This also applies to *Exobasidium rhododendri* Cramer, a parasite on *Rhododendron ferrugineum* and *Rhododendron hirsutum*. However, according to this author, *Exobasidium pentasporium* Shir., which occurs on *Azalea indica* in Japan but not in Europe, seems to be another good species. Savile (1959), who studied the morphology of many *Exobasidium* species, is also of opinion that several parasites belonging to the genus *Exobasidium* have erroneously been described as new species. Several of them are identical. It is not excluded, however, that some of them may be regarded as formae speciales occurring on different host plants. Only experiments can give an answer to the question whether specialization exists or not. In this investigation cross inoculations were performed with *Exobasidium* spp. originating from Azalea and from *Vaccinium vitis idaea*.

4.2. Description of the fungus on azalea

The mycelium is intercellular. The width of the hyphae, $\pm 1\ \mu$, depends on the width of the intercellular spaces. The mycelium forms a subepidermal layer. The apices of the hyphae, the future basidia,

Fig. 1. Basidia (b) developing basidiospores (bs) on sterigmata (s). One of the basidiospores forms a blastospore (bl) in situ.

stretch in a direction perpendicular to the epidermis, thus forming the hymenium. By further growth the basidia find their way between the epidermal cells and rupture the cuticle. The basidia are cylindrical, 24–30 × 5–8 μ. The basidiospores develop on sterigmata, 4 to 5 on one basidium. The size of the basidiospores is 13–18 × 2.5–5.5 μ (Fig. 1). By budding the basidiospores produce a large number of spores, measuring 9–13 × 2–4 μ. They are designated as "conidia" by Woronin. According to MIX (1949), an analogy exists between the behaviour of the ascospores of the genus *Taphrina* and the basidiospores of the genus *Exobasidium*, in so far that both may produce new spores by budding. Mix uses the name "blastospores" for these products, which reminds one of the outgrowths formed by yeast cells. Among the *Protobasidiomycetes* too many forms occur, e.g. the smut fungi, in which the basidiospores produce new spores by budding. In this case the newly formed spores are called "sporidia" or "secondary sporidia". In this paper the yeast-like spores of *Exobasidium* will be called "blastospores".

4.3. ANATOMY OF THE GALLS ON AZALEA

Not only macroscopically (p. 351), but also microscopically a sharp boundary is seen between the healthy and the diseased tissue, when a gall is studied in transverse sections. There is a lack of differentiation in the mesophyll of the hypertrophic part, and tracheal elements are but sparingly found in the uniform tissue. In comparison with the

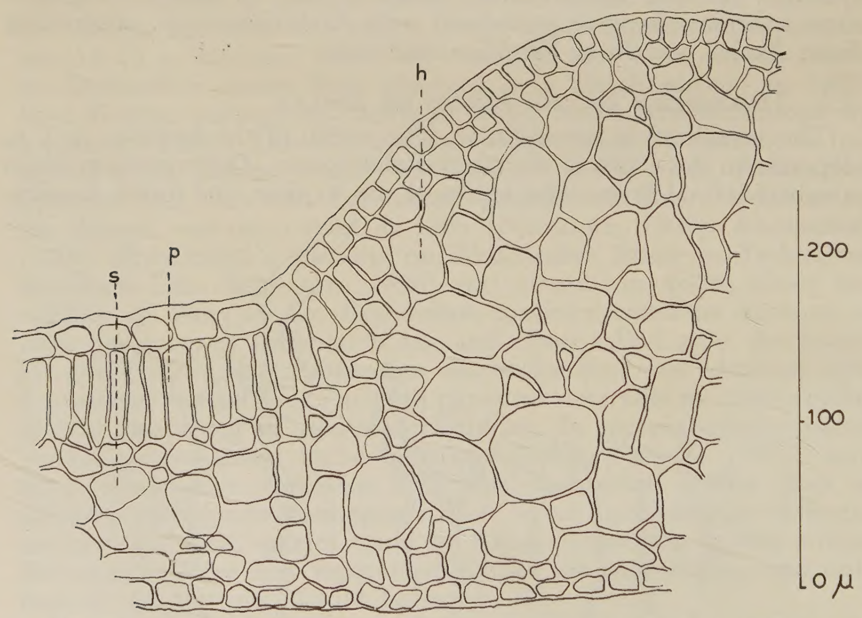


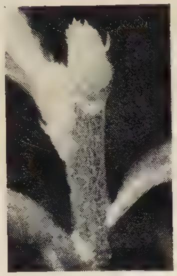
Fig. 2. Transverse section through an infected leaf, showing a healthy part with palissade parenchyma (p) and spongy parenchyma (s), and a diseased part with hypertrophic cells (h).

PLATE

A

B

C



D

E

F

G

Plate 1. A. *Azalea obtusa*, cultivar 'Esmeralda', with galls of *Exobasidium japonicum* Shir. B. *Azalea indica*, cultivar 'Hexe', with galls of *Exobasidium japonicum* Shir. C–G. Development of axillary buds of *Azalea indica*, cultivar 'Hexe', after decapitation of the shoot. C: just after decapitation; length of the buds about 2 mm. D–G: further developmental stages; length of the buds in D: 4 mm; in E: 6.5 mm; in F: 9 mm and in G: 12 mm.

normal mesophyll, the number of cells as well as their size have increased: both hyperplasy and hypertrophy occur (Fig. 2).

This same anatomical picture has been observed by several other authors (PETRI, 1907; NAUMANN, 1909; MARCHIONATTO, 1929 and LAUBERT, 1932).

The epidermal cells, however, are not enlarged, though their number is abnormally increased. They keep their healthy appearance until the epidermal layer is burst by the basidia.

In galls which develop on the stems, differentiation of the tissues is also lacking. The anatomical features are similar to those observed in the leaf.

CHAPTER V

THE MATERIAL USED FOR THE EXPERIMENTS

5.1. The plant material

Of the Japanese Azaleas grown in Boskoop three cultivars were used, viz. 'Esmeralda', 'Galestin' and 'Moederkensdag', with pink, white and red flowers respectively.

The cultivar 'Hexe' was obtained from Ghent (Belgium). Because of its excellent vegetative growth and flowering, this variety is grown frequently in the Belgian nurseries. An unfavourable characteristic, however, is the susceptibility of this variety to the *Exobasidium* disease.

Specimens of *Vaccinium vitis idaea*, for use in cross inoculation tests were gathered in nature and grown in the garden of the laboratory.

The Azaleas were multiplied by cuttings. Young, recently hardened shoots of about 8 cm length were cut either in April, while the plants were still in the glasshouse, or they were cut in July or August, while growing out of doors. The cuttings were kept in the glasshouse under double glass-cover. The soil was composed of a mixture of 1/3 leaf mold, 1/3 peat moss and 1/3 sand. Roots developed in 4–6 weeks. A treatment with growth substance to stimulate root formation was not necessary. Very favourable conditions for rooting were obtained by the use of clay seedpans and by keeping the humidity high.

The rooted cuttings were potted in the same soil mixture after some stable manure had been added. After some weeks the tips of the shoots were removed, resulting in the unfolding of the axillary buds. Small plants were obtained with 3 to 4 branches which were suitable for the experiments.

5.2. The isolation and cultivation of *Exobasidium japonicum*

Woronin observed the germination of basidiospores of *Exobasidium vaccinii* as early as 1867. Under humid conditions they sprouted in a yeast-like way, forming "conidia". BREFELD (1889) grew the fungus in pure culture in a liquid medium, in which the cells multiplied by sprouting. RICHARDS (1896), however, reported that his attempts to grow the fungus in pure culture, had failed. LOCKHART (1958)

obtained a pure culture of this *Exobasidium* by pouring out a suspension of basidiospores on agar.

MARCHIONATTO (1929) was the first to report the growth of *Exobasidium japonicum* in pure culture. The "conidia" developed on potato-agar. EZUKA (1955) grew *Exobasidium japonicum* from *Rhododendron obtusum* var. *kaempferi* and *Exobasidium vexans* from *Thea sinensis* in pure culture.

GRAAFLAND (1953) succeeded in growing 4 species of *Exobasidium* in pure culture, viz.:

Exobasidium vaccinii Wor. from *Vaccinium vitis idaea*; *Exobasidium rhododendri* Cramer from *Rhododendron ferrugineum* and *Rhododendron hirsutum*; *Exobasidium japonicum* Shir. from *Azalea indica*; *Exobasidium vexans* Massee from *Thea sinensis*.

The following method to grow the fungus in pure culture appeared to be the most suitable one: Young, newly sporulating galls were gathered from the host plants. They were placed in sterile petri dishes, where humidity was kept high by the presence of a plug of wet cotton wool. The galls keep well in this way. If shoots with hypertrophic leaves are used, the galls can be kept fresh for an even longer time by wrapping the cut surface of the shoot in wet cotton wool. The sporulating surfaces of the galls have to be turned as much as possible towards the bottom of the petri dish, in order to allow the forcibly expelled basidiospores to fall on the glass, where they start germinating. Even within 24 hours so many blastospores are formed that they can be taken off with a needle and transferred to a nutrient agar. After 6 to 8 days small, hemi-spherical, glassy colonies are formed with a shiny surface. Gradually they develop the appearance which is typical of *Exobasidium* colonies, i.e. with a sunken centre and peripheral growth. The surface of the mycelial mat gradually shrivels and becomes wrinkled like the surface of a brain. On most of the nutrient media used the colour darkens from yellow to brown. Malt and malt-saleb agar are most favourable for the growth of *Exobasidium* cultures. A temperature of 20–22° C is optimal. The fungi were subcultured every two months.

It is also possible to grow *Exobasidium japonicum*, *Exobasidium vaccinii* and *Exobasidium rhododendri* in nutrient solutions instead of on agar. Shaking is necessary, otherwise the development of the fungus does not proceed beyond the formation of the mycelial mat. With good aeration in a shake culture the fungal particles sprout throughout the solution. This results in a finely divided suspension consisting of cells of irregular shape. A malt solution is most favourable for obtaining such a culture.

Microscopically the length of the hyphae grown on agar varies from 13 to 30 μ, depending on the nutrients used. The width of the hyphae is 1–2 μ. Their shape is irregular, but part of them are fusiform, in which case they are similar to the blastospores formed on the galls of the plants. The particles may also be rectangular or crooked with a knob on one or both ends. Sometimes, especially in old cultures, threads are formed, though the latter can never be

considered to be real hyphae, as the cells are only loosely attached to each other (Fig. 3).

In the nutrient solutions the cells are more uniform; cells cohering in threads are hardly ever found. Though the cells are comparable to the typical *Exobasidium*-blastospores formed on the galls, they are more irregularly shaped. Especially their length is more variable.

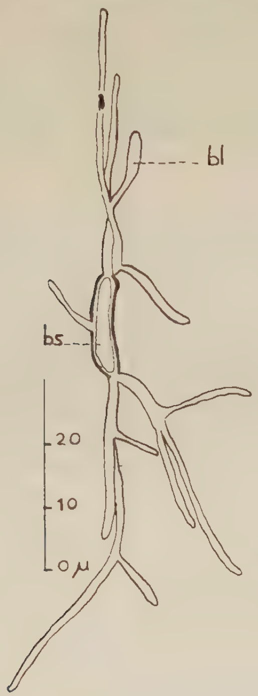


Fig. 3. Germinating basidiospore (bs), forming hyphae and blastospores (bl).

CHAPTER VI

INOCULATION EXPERIMENTS

6.1. Literature

Inoculations with basidiospores taken directly from sporulating galls were performed already by Woronin (1867), who succeeded in infecting healthy plants of *Vaccinium vitis idaea* by means of basidiospores obtained from diseased ones. Richards (1896) obtained a similar result with *Andromeda ligustrina* and *Gaylussaccia resinosa*. Lockhart (1958) observed typical disease symptoms on the young leaves of shoots developing on *Vaccinium myrtilloides* and on *Vaccinium angustifolium*, three months after these plants had been treated with basidiospores. Graafland (1953) inoculated young tea plants with basidiospores of *Exobasidium vexans*, and obtained good results. No information is available with regard to inoculations of Azalea carried out with basidiospores taken from the galls.

Inoculations with blastospores obtained in pure culture were performed by BREFELD (1889). He states, though not very clearly, that the spores of *Exobasidium vaccinii* obtained in pure culture and those formed by sprouting basidiospores have similar effects on *Vaccinium vitis idaea*. GRAAFLAND (1953) obtained good results by inoculating this plant with cells taken from a pure culture of the parasite. Similar results were obtained by LOCKHART (1958) after inoculating *Vaccinium myrtilloides* and *Vaccinium angustifolium* with blastospores of the same fungus. There was no difference between the results obtained with inoculations by means of basidiospores originating from the plants and those obtained with cells grown in pure culture.

6.2. EXPERIMENTS

Well-rooted and vigorously growing cuttings were used for the inoculations. As the galls are already visible when the leaves are a few mm in length it was clear that inoculation had to be performed before the buds began to expand. After the inoculation, however, the dormancy of the buds had to be broken. In order to achieve these two ends axillary buds were inoculated, and the tips of the stems were removed. As, usually, not more than 4 buds per shoot began to expand after the removal of the tip, only the 5 uppermost buds were treated with inoculum. If the plants were too small to be decapitated, the terminal bud was inoculated, although this was more difficult than inoculating an axillary one. The latter consists of a growing point surrounded by only 4 to 6 diminutive leaves, of which 4 are bud-scales. The terminal bud is covered by about 10 of these leaves in different developmental stages. It is not easy to inoculate these tightly packed small organs. Basidiospores and also blastospores, if present, were transferred from diseased plants to healthy ones by means of a moist brush. The inoculated plants were kept for 24 to 30 hours in glass inoculation-chambers, which on the inside were covered with moist filter paper. Then the chambers were opened, and the filter paper was removed. The first symptoms were visible after about 3 weeks, at least if the temperatures had not fallen too much at night. When the nights had been cool, i.e. in September when the temperature could be as low as 0°–5° C, the incubation period was sometimes much longer than 3 weeks.

The number of galls obtained after inoculation was much larger when the plants were decapitated than without this treatment. (Table 1).

TABLE 1

Number of galls on groups of 5 plants inoculated with basidiospores after decapitation, and inoculated without decapitation.

Azalea cultivars	number of galls	
	shoots decapitated	shoots not decapitated
'Esmeralda'	4	0
'Galestin'	14	3

Thus the development of the axillary buds appeared to be favourable for the development of the symptoms. The technique of inoculation was greatly simplified when it was discovered that inoculations could successfully be performed with pure cultures, especially with suspensions from a shake culture. When the culture was 14 days old, the shaking was suspended during one night in order to give the cell material the oppertunity to sink to the bottom of the flask. After discarding the supernatant fluid, the viscose suspension was used as inoculum by brushing it on the buds of the plants. For the first experiment the cultivars 'Esmeralda' and 'Galestin' were used. Half the number of plants were inoculated with basidiospores originating from a gall, and the others were treated with a suspension of the pure culture.

The number of galls which developed on the latter group was even larger than the number that developed on the plants treated with basidiospores (Table 2).

TABLE 2

Number of galls that developed on groups of 5 plants treated respectively with basidiospores and with suspensions of cells grown in a pure culture.

Azalea cultivars	inoculated with	
	basidiospores	a cell suspension
'Esmeralda'	4	5
'Galestin'	7	9

After such favourable results had been obtained with suspensions, all further experiments were performed with material grown in shake cultures.

Cultures of *Exobasidium japonicum* and *Exobasidium vaccinii* isolated in 1952 have remained virulent up to now, as after inoculation host plants become infected.

CHAPTER VII

THE LIFE CYCLE OF *EXOBASIDIUM* SPECIES

7.1. LITERATURE

The mode of infection of *Exobasidium vaccinii* was revealed by the brilliant observations of Woronin. The basidiospores proved to germinate on the leaves of *Vaccinium vitis idaea*. Sometimes secundary "conidia" were formed by budding, but usually the spores themselves formed germ tubes, which penetrated via the stomata or via the epidermal cells. In the intercellular spaces the branching hyphae developed into a network within 48 hours after the infection.

According to HILBORN & HYLAND (1956) the infection of *Vaccinium myrtilloides* and of *Vaccinium angustifolium*, the "lowbush blueberry", by *Exobasidium vaccinii* occurs via the cuticle of the stem. The infection becomes systemic, and the mycelium is perennial in the rhizomes. Much attention has been paid to the way in which the tea plant

becomes infected by *Exobasidium vexans* Massee (TUBBS, 1947; GADD & LOOS, 1948, 1949 and 1950; REITSMA & VAN EMDEN, 1949 and 1950; LOOS, 1951). This parasite can attack even the 3rd or the 4th leaf reckoned from the tip of the stem, i.e. the leaf which is just fully developed, and also the leaves of a developing bud. The basidiospores of this parasite do not form blastospores, but they develop a germ tube. From an appressorium on the cuticle a hypha may penetrate the outer epidermal cell wall.

Only little is known with regard to the way in which Azaleas are infected by *Exobasidium japonicum*. According to MARCHAL (1925) and MARCHIONATTO (1929) the germ tubes penetrate into the young leaves during the unfolding of the buds.

EFTIMIU & KHARBUSH (1927) studied the cytology of *Exobasidium* species extensively. They studied the spores, the mycelium, and the basidia of an *Exobasidium* occurring on Azaleas grown in the vicinity of Paris. Though these authors considered this fungus to be *Exobasidium discoideum* Ellis, it can be concluded, especially from their illustrations, that it was *Exobasidium japonicum* Shir. According to these authors, the basidiospores of this fungus are monocaryotic, whereas the cells of the intercellular mycelium are dicaryotic. Also in the young basidia two distinct nuclei are present, which fuse to a diploid one. After two divisions the latter gives rise to the 4 haploid nuclei of the 4 basidiospores.

7.2. EXPERIMENTS

It was not yet known in what stage of the life cycle the diploidization occurs, of which the result is recognizable in the dicaryotic condition of the mycelium found in the leaves. To answer this question we had to know first of all whether the basidiospores, and also the blastospores really are uninucleate. Secondly, the possibility of a cell fusion outside the host plant had to be investigated. For that purpose basidiospores from a mature gall caught on a glass slide were allowed to germinate, after which the nuclei were stained with haematoxylin. The basidiospores, as well as the blastospores developed out of the former, appeared to be uninucleate (Fig. 4 and 5). The possibility of a fusion between these spores was studied in a moist chamber culture. A small piece of a mature gall was glued to the bottom of the chamber inside the glass ring, with the sporulating side directed towards the cover slip; on the latter a drop of malt agar had been placed (5 % malt in 1 % agar). By keeping the moist chamber upside down for several hours, we obtained that the basidiospores collected on the agar. Nearly every spore germinated, which could be observed microscopically. The contents of the spores moved towards the 2 poles, after which germination of the spores took place on either side, resulting in the formation either of 2 blastospores or of 2 hyphae each bearing a terminal blastospore. In the meantime the spore itself became divided by one or more septae. No sign of cell fusion was ever observed: neither were basidiospores lying in the neighbourhood of each other, nor blastospores originating from different basidiospores

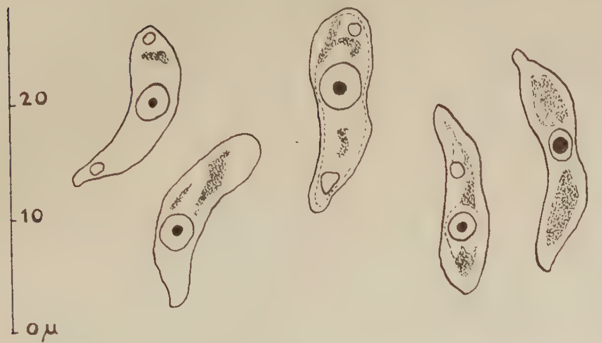


Fig. 4. Basidiospores with nuclei stained with haematoxylin.

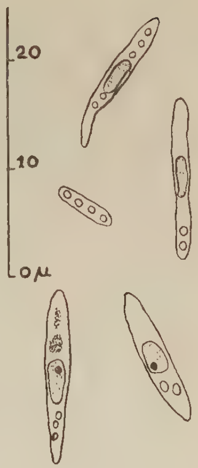


Fig. 5

Fig. 5. Blastospores developed in a shake culture, stained with haematoxylin.

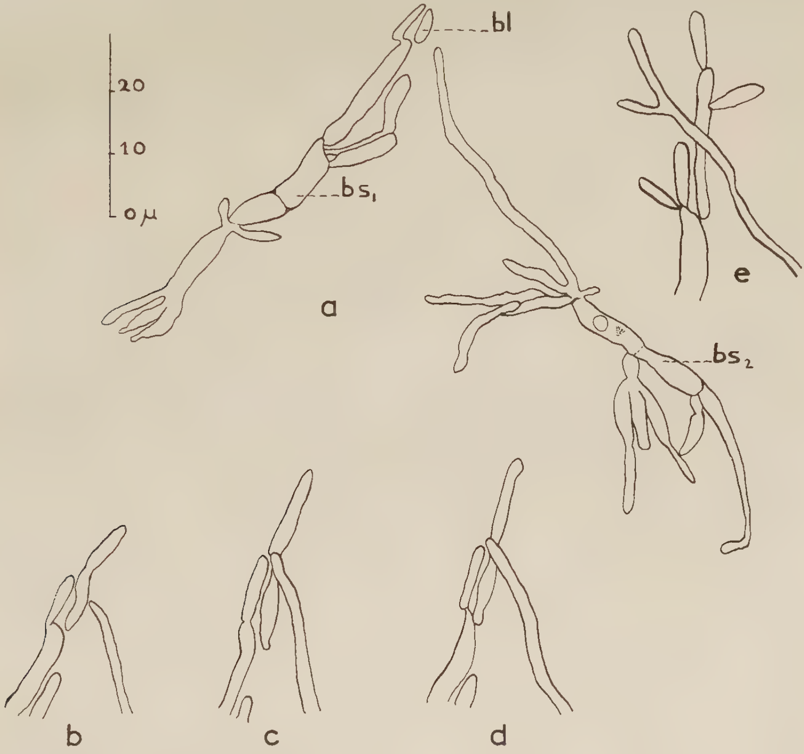


Fig. 6. *a*: Two germinating basidiospores (bs_1 and bs_2) originated from one basidium, forming hyphae and blastospores (bl). *b*, *c*, *d* and *e*: Blastospores and tips of hyphae of *a*, after 5, 10, 29 and 45 hours, respectively.

ever seen conjugating even though they were separated from each other only by a small distance. The colonies of blastospores grew against each other without making contact (Fig. 6). In many moist chamber cultures the basidiospores were found in groups of 4 or 5, probably originating from the same basidium. If + and — spores existed, it might be expected that both would be represented in these groups. Therefore, special attention was paid to these cases, but even here no fusion was observed. It may be concluded that in vitro all spores behave in a similar way.

Another possibility that had to be investigated, is that the dicaryotic mycelium in the young leaves develops after a union of the spores or of the germ tubes either on the surface of the leaves or after penetration. If the fungus were heterothallic, at least one + and one — spore would be required to accomplish an infection. In case the fungus were homothallic, only one spore would be necessary for the development of a gall. To investigate this question it was necessary to dispose of monospore cultures.

Monospore cultures were obtained from a suspension of a shake culture by diluting the latter so far that a drop taken on a needle contained only one cell. Such a drop was placed on the malt agar sticking to the cover slip of a moist chamber culture. After it had been ascertained by microscopic examination that only one spore was present, the latter was allowed to develop into a colony. After one to two weeks the colony was transferred to malt agar. In this way about 10 monospore cultures were obtained. Each one was tested on a group of 5 test plants as to its ability to cause infection. Five of these cultures proved to cause infection on all 5 plants, 2 of them caused infection on 4 of the group of 5 plants and 3 monospore cultures caused galls on 3 out of a group of 5 plants. Thus it may be concluded that the fungus is homothallic, because sporidial cells derived from a single blastospore are able to develop into a dicaryotic mycelium.

Monobasidiospore cultures were obtained in moist chamber cultures as described before (p. 360). The time during which the chamber had to be kept upside down, however, was now but short. A period of one to two minutes appeared to be most suitable to obtain basidiospores separated from each other by such a distance that the colonies into which they developed did not touch. This was controlled microscopically. If more than 10 basidiospores were present on the cover slip, the culture was discarded. In many cultures only one basidiospore was present, in others 2 to 5. In three cases it was possible to isolate the spores of such a group and to raise separate colonies from them. All in all 34 monobasidiospore cultures were obtained, among which 21 originated from basidiospores belonging to different basidia. In two instances it was possible to grow a colony out of each of 4 basidiospores which probably originated from the same basidium. Once, 5 colonies were obtained from 5 basidiospores which were probably derived from the same basidium. These cultures were tested separately on Azalea plants. They all caused infection (Table 3).

TABLE 3

Number of plants developing galls after inoculation with monobasidiospore cultures

number of monospore cultures; each one was used to inoculate 5 plants		numerator: number of plants with galls denominator: number of plants inoculated
9		45/9 × 5
6		24/6 × 5
6		18/6 × 5
2	basidiospores originating from one basidium	10/2 × 5
1		4/1 × 5
1		3/1 × 5
1	the same	5/1 × 5
3		12/3 × 5
2	the same	10/2 × 5
1		4/1 × 5
2		6/2 × 5

These experiments prove that *Exobasidium japonicum* is homothallic. Monocaryotic cells, formed by a process of budding out of one basidiospore and, therefore, genotypically identical, penetrate the outer wall of the epidermis of the young leaves. In the leaf tissue the dicaryotic mycelium, therefore, is formed out of cells between which there can be no difference in sex.

CHAPTER VIII

CROSS INOCULATIONS

8.1. CROSS INOCULATIONS WITH *Exobasidium* SPORES ORIGINATING FROM DIFFERENT AZALEA CULTIVARS

The *Exobasidium* galls are found on the Japanese *Azalea obtusum* varieties grown in Boskoop as well as on the cultivars grown in Ghent, which have *Rhododendron simsii* as ancestor. The latter seem to be very susceptible to the disease. It is as yet uncertain whether these galls are caused by two different parasites, each occurring on a different group of cultivars or by only one parasite, causing galls in all cultivars. Morphologically no differences could be found between the shape of the galls on the different cultivars, and the basidiospores too proved to be identical, measuring 13–18 × 2.5–5.5 μ. However, physiological specialization might occur. To study this question, cross inoculations were performed with basidiospores as well as with cell suspensions from shake cultures, the one like the other originating from infected plants of the Japanese cultivars 'Esmeralda' and 'Galestin', and from infected plants of the Belgian cultivar 'Hexe'. Table 4 shows that the parasite from the Japanese Azalea could cause galls on the Belgian cultivar and vice versa. The Japanese cultivars could be infected with the fungus from 'Esmeralda'. The parasite from 'Hexe' easily caused infections on plants of the same cultivar, but

TABLE 4

Number of galls developing on different Azalea cultivars after inoculation with *Exobasidium* of different origin.

Azalea cultivar	origin of the *Exobasidium* inoculum	number of galls / number of plants [1]	number of diseased plants
'Esmeralda'	'Hexe'	16/6	
'Galestin'	'Hexe'	25/6	
'Esmeralda'	'Esmeralda'	37/6	
'Galestin'	'Esmeralda'	36/6	
'Esmeralda'	control	0/6	
'Galestin'	control	0/6	
'Hexe'	'Hexe'	76/20	19
'Esmeralda'	'Hexe'	46/20	17
'Hexe'	'Esmeralda'	42/20	19
'Esmeralda'	'Esmeralda'	31/20	12
'Hexe'	control	0/20	0
'Esmeralda'	control	0/20	0

[1]) numerator: total number of galls; denominator: number of inoculated plants.

was less apt to provoke galls on the Japanese cultivars. Conversely, the fungus from the latter was able to cause infections on 'Hexe', even to a higher degree than on the cultivars from which it originated.

Though some specialization seems to occur, *Exobasidium japonicum* is certainly not narrowly specialized on certain Azalea varieties, as the parasite may attack Azaleas of different genetic composition.

8.2. CROSS INOCULATIONS WITH *Exobasidium* SPORES ORIGINATING FROM DIFFERENT LESS CLOSELY RELATED HOST PLANTS

According to SAVILE (1959) and other authors, the *Exobasidium* species occurring on Azalea are identical with *Exobasidium vaccinii* described by Woronin. This opinion is based on the morphological similarity of these fungi. However, even if they are considered to be a single species, this does not mean that the *Exobasidium* strains occurring on different host plants might not be specialized in their pathogenicity. If they are different physiological races or biotypes they may be expected to occur on different host plants. To investigate the parasitic character of some *Exobasidium* isolates from different origin, cross inoculations were performed with pure cultures obtained from the Belgian Azalea cultivar 'Hexe', from the Dutch cultivar 'Esmeralda' and from *Vaccinium vitis idaea*. GRAAFLAND (1953) previously succeeded in obtaining infections of the latter by inoculating it with a pure culture of *Exobasidium vaccinii*.

Cross inoculations in the experiments described in this paper were performed with pure cultures of *Exobasidium japonicum* obtained from different Azalea cultivars and of *Exobasidium vaccinii* obtained from *Vaccinium vitis idaea*.

Pure cultures of both fungi on agar showed a different aspect. The brown colonies of the former produced a dark discolouration

of the nutrient agar. The light yellow colonies of the latter hardly changed the colour of the agar. Both parasites also showed a difference in colour of the nutrient solution when grown in shake cultures.

From cross inoculations it became clear that *Vaccinium vitis idaea* was not attacked by the *Exobasidium* from Azalea, and that conversely Azalea does not function as a host plant to the fungus living on *Vaccinium*. In all 6 sets of experiments were made, all with similar results. The results of one of these sets of experiments are given in table 5.

TABLE 5

Number of galls resulting from inoculation of Azalea and *Vaccinium vitis idaea* plants with pure cultures of *Exobasidium* of different origin; 20 test plants in each set.

origin of the pure culture	test plants	number of galls	number of diseased plants
	Azalea cultivars:		
Exobasidium japonicum	'Esmeralda'	25	17
,, ,,	'Hexe'	40	19
,, ,,	*Vaccinium vitis idaea*	0	0
	Azalea cultivars:		
Exobasidium vaccinii	'Esmeralda'	0	0
,, ,,	'Hexe'	0	0
,, ,,	*Vaccinium vitis idaea*	22	15

Despite their morphological similarity, there is a difference in pathogenicity between these fungi. It is possible, therefore, to consider them to represent physiological races of one species, viz. *Exobasidium vaccinii* Wor. If, however, the physiological specialization of the fungus and the difference in aspect of the pure cultures are considered to be of sufficient importance to distinguish more species, then the valid name for the fungus on Azalea would be *Exobasidium japonicum* Shir. This standpoint is taken up in the present paper, at least if we assume that the American species *Exobasidium azaleae* Peck and *Exobasidium discoideum* Ellis are different too.

It is regrettable that the set of cross inoculations carried out with *Exobasidium* cultures originating from Azalea and from Swiss *Rhododendron* species remained incomplete, as specimens of the latter were not available. *Exobasidium rhododendri*, parasite of the Swiss *Rhododendron ferrugineum* was obtained in pure culture (GRAAFLAND, 1953). On malt agar this fungus differs from *Exobasidium vaccinii* Wor. and *Exobasidium japonicum* Shir. in developing colonies with a more finely wrinkled surface and with a grey-brown rather than a yellow or brown colour. 'Hexe' was inoculated with this pure culture, but no galls appeared. Though there was no opportunity to inoculate *Rhododendron ferrugineum* with *Exobasidium* from 'Hexe', and the evidence, therefore, is not complete, it may be assumed that the fungus from this *Rhododendron* must also be considered as a physiologically specialized form.

CHAPTER IX

DEVELOPMENTAL STAGES OF THE BUD AT WHICH INFECTION WITH *EXOBASIDIUM JAPONICUM* IS POSSIBLE

Efforts were made to obtain more information concerning the developmental stages of the young leaves which are sensitive to infection by this fungus. For that purpose dormant axillary buds of Azalea shoots were inoculated with an *Exobasidium* suspension immediately after the shoots had been decapitated and the buds had started to develop. At different intervals after inoculation the axillary buds were removed and fixed in Craf's solution. In this way the developmental stages of the young leaves and the progress of the gall formation could be studied 0, 1, 2, 3, 4, 5, 7, 10, 14, 21 and 28 days after the inoculation.

Microtome slides revealed that within 4 days after the inoculation the infected young leaves showed hypertrophy and hyperplasy. All differentiation between the palissade layer and the spongy parenchyma proved to be lacking. In a few cases hyphae were visible in the intercellular spaces, though the process of penetration itself was not observed. The small size of the blastospores made it difficult to detect them between the hairs and the scales which cover the epidermis of the young leaves. However, it became clear that the young leaves are already sensitive to infection when they are still enclosed in the bud.

The aim of the following experiment was to study the length of the period during which the young leaves could still be infected. For

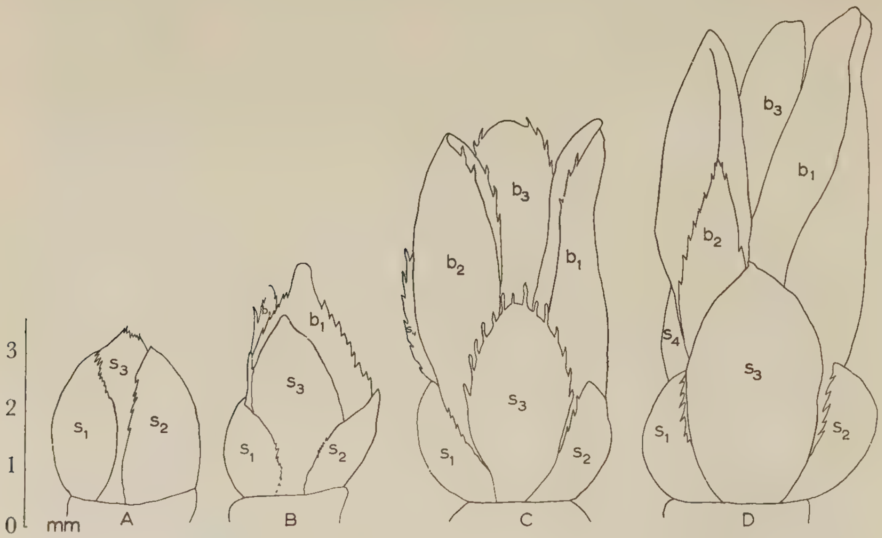


Fig. 7. A–D: development of an axillary bud of *Azalea indica*, cultivar 'Hexe'. b_1-b_3: leaves. s_1-s_4: bud scales.

that purpose in 70 Azalea plants the tips of the shoots were removed in order to stimulate the unfolding of the buds. At different intervals after this unfolding, the buds were inoculated with a pure culture of *Exobasidium*. Ten plants were used for each set of inoculations. It was found that leaves of 8–10 mm length still could be infected. In the next stage of development the laminae begin to spread out, and in that stage the leaves are no longer susceptible to infection (Table 6; Fig. 7 and Plate 1, C–G).

TABLE 6

Number of galls on the leaves of axillary buds inoculated in different developmental stages i.e. at different moments after the decapitation of the shoots.

number of days after decapitation	length of the axillary buds in mm	number of galls per group of 10 plants	number of plants with galls
0	1.5	70	10
11	2.5	85	10
19	3.0	30	9
25	4.0	17	8
30	6.5	42	10
40	8–10	1	1
50	12–15	0	0

The stage of development at which a leaf is still subject to infection, was studied also in another way. The young just visible leaves of 40 plants were inoculated with a pure culture of *Exobasidium*; the 4th visible leaf reckoned from the tip of the shoot being the last to be inoculated. The inoculated leaves were marked with paint, at least when they were not too small for this treatment. After 5 weeks 42 galls had appeared on those leaves which had developed after the inoculation. Twelve galls were present on those leaves which at the time of inoculation were about 8 mm in length and just large enough to be marked. This result is in agreement with that of the former experiment: the leaves are only susceptible to infection in a juvenile stage. As soon as the laminae begin to spread out, infection is no longer possible.

From a practical point of view it was especially important to answer the question how long inoculum of *Exobasidium* remains viable on or in dormant buds. In the greenhouses the Azaleas may already be infected by the spores of *Exobasidium* galls long before the growers start cutting or pruning, and, therefore, long before the dormancy of the axillary buds is broken. An experiment was performed with artificially administered inoculum. Nine groups, each of 5 plants, were inoculated with a pure culture. In each plant the youngest visible leaf was marked with paint. On every shoot the 4 axillary buds inserted below the marked leaf were inoculated. The plants were then kept at high humidity for 30 hours. At different intervals, up to 4 weeks after the inoculation, the tips of the shoots were removed just above the marked leaf to stimulate the development of the buds. It appeared that even buds inoculated 4 weeks before they began to

expand, showed gall development (Table 7, experiment 1). The experiment was repeated in the same way, but instead of removing the tips of the shoots within a period of at the most 4 weeks after the inoculation, longer intervals were chosen. Even 6 months after the inoculation of the dormant buds the fungus still appeared to be viable, for galls were found in buds that remained dormant for half a year after they had been treated with the suspension (Table 7, experiment 2).

Table 7

Number of galls occurring on young leaves developing at different intervals after inoculation with an *Exobasidium* suspension; five plants in each group.

	Number of days after the inoculated buds begin to expand	number of galls	number of plants with galls
experiment 1	0	17	5
	2	20	5
	3	18	5
	5	16	5
	9	10	4
	12	7	5
	18	3	3
	23	4	3
	30	4	3
experiment 2	14	14	4
	24	11	5
	28	5	3
	35	11	4
	56	2	2
	91	3	3
	119	2	2
	182	4	2

These experiments shed no light on the question how the fungus behaves during the period between the inoculation and the development of the gall, and how it obtains the necessary nutrients. It is possible that the blastospores administered to the buds start budding on their surface, and that the colonies thus formed remain viable between the hairs and the scales of the dormant buds. Penetration of the young leaves may occur at the time the buds begin to expand. However, it is also possible that the spores penetrate directly into the leaves of the dormant bud, and that the development of the hyphae inside the latter is delayed as long as the buds remain dormant. Which of these two pathways actually is taken, remains undecided as the first developmental stages of the disease could not be detected. The rate of development of the host plant determines the time at which the symptoms of the disease will appear. If infected axillary buds are stimulated to new growth, a parallel development of host and parasite takes place. Thus the incubation period depends on the environmental conditions: if the buds are just unfolding at the time

of the contamination, the symptoms may appear within twenty days after the infection but they may also be delayed for 6 months or longer after the infection, if the buds remain dormant for that period.

CHAPTER X

THE CONTROL OF THE DISEASE

10.1. Hygienic measures

In the literature the importance of removing and burning the galls has frequently been pointed out (Naumann, 1909; Miles, 1928; White, 1933; Stautemas, 1951; Jaenichen, 1954). This is sometimes done in combination with spraying with Bordeaux mixture. The "Tuinbouwgids" of 1960 (Dutch Horticultural Guide) still recommends these measures, though it is known that they are not conclusive (Welvaert, 1952; Graafland, 1957). This method would be satisfactory if the galls were removed in time, i.e. before sporulation, when the colour is still green, but the picking of mature galls promotes the spread of the basidiospores. However, it is impractical to remove all gall bearing leaves sufficiently early from large numbers of densely planted Azaleas. Moreover, before they begin to expand, infected buds cannot be distinguished from healthy ones. Thus a continual control of the unfolding buds would be necessary. Even if the fungus were eradicated in this way from a greenhouse, a new contamination could easily occur from spores blown in from neighbouring places.

It is obvious that after removing the galls only once, the plants may still be diseased. Plants imported from Belgium in an apparantly healthy state may show galls at the time the dormant buds are unfolding. For that reason *Exobasidium japonicum* may easily be introduced into other countries, because it may be hidden in dormant buds of imported Azalea plants. In this manner it is easy to defeat the quarantine laws, which list the fungus as a dangerous parasite and forbid its importation.

10.2 Control by fungicides

In the literature spraying with fungicides has frequently been advised. Marchal (1925) recommends the use of lime after pruning; Miles (1928) and Marchionatto (1929) prefer Bordeaux mixture, though Marchionatto also mentions lime. Cole (1944) and Thiel (1951) also consider Bordeaux mixture to be an effective spray; Thiel recommends the use of lime sulphur. Ciferri (1953) mentions zincethylenebisdithiocarbamate as a good preventive fungicide. Jaenichen (1954) prefers a treatment with carbamates following the removal of the galls. Anonymus (1956) had good results by spraying with copperoxychloride or zineb after the galls had been removed as far as possible.

Many authors consider insects to be effective in transmitting spores from mature galls to other parts of the plants. For that reason the control of insects is important (Stautemas, 1951). Next to insects

mites might be involved, and these animals too should, therefore, be killed (MILES, 1928; MARCHIONATTO, 1929). Marchionatto based this opinion on his observation of basidiospores attached to the bodies and the mouth parts of these arthropods. MARCHAL (1925) regards *Aleurodes vaporarius* as causing the spread of the spores. Though it is possible that insects and mites play a rôle in the transfer of *Exobasidium* spores from galls to healthy parts of the plants, the disease is in our opinion mainly spread by water droplets carried by draughts arising when the glasshouses are aired and augmented by spray irrigation. In whatever way contamination occurs, protection of the buds with a fungicide is necessary. Fungicides have to be applied regularly, and not alone before or after pruning. Some growers even spray every fortnight with copper compounds, and succeed in this way in keeping their plants free from the *Exobasidium* disease as well as from other parasitic fungi, such as *Septoria azaleae* Vogl. A drawback is that the plants are more or less severely damaged by such frequent sprayings. They shed their leaves, and become unattractive for sale. Spraying during the period of flowering is disastrous.

10.3 EXPERIMENTS WITH FUNGICIDES IN VITRO

In the "Tuinbouwgids" of 1960 (Dutch Horticultural Guide) copper compounds or lime sulphur are advised for spraying. As these fungicides are more and more going out of use, some of the newer compounds, i.e. zineb and captan, were assayed against the *Exobasidium* disease.

The irregularly shaped blastospores developing in a shake culture can be obtained in large numbers, but they can hardly be used in the ordinary germination tests in vitro, in which the number of germinating spores is counted, as only a small percentage are budding. It is also impossible to assess the E.D. 50 of the basidiospores, as they do not germinate at all in watery suspensions. Budding only occurs when the spores are covered with a thin film of condensation-water, i.e. when kept at a high humidity. Even if basidiospores could be used in assaying, hundreds of Azaleas would have to be inoculated in order to obtain the high numbers of spores needed for the experiments. To avoid these difficulties, different concentrations of the fungicides were added to the nutrient solution of shake cultures. The inhibition of the growth of the blastospores could be determined by weighing the mycelium. The following fungicides were used in concentrations varying from 1/10 to 1/100 of those used in practice:

copperoxychloride:	0.05,	0.10,	0.20 and 0.50	g/l
lime sulphur:	0.075,	0.15,	0.30 and 0.75	cc/l
zineb [1])	0.025,	0.05,	0.10 and 0.25	g/l
captan [2])	0.02,	0.04,	0.10 and 0.20	g/l

The experiments were carried out in erlenmeyer flasks containing

[1]) zincethylenebisdithiocarbamate.
[2]) 4, 5– cyclohexene –1, 2– dicarboximide.

50 cc of a malt solution to which known quantities of water and of a standard solution had been added. The media containing copperoxychloride were prepared as indicated in table 8.

TABLE 8

Composition of the media containing copperoxychloride.

malt solution in cc	water in cc	copperoxychloride	
		quantity used of a 0.1 % standard solution in cc	concentration in g per l
50	45	5	0.05
50	40	10	0.1
50	30	20	0.2
50	0	50	0.5

The other media were prepared in a similar way. Thus solutions were obtained equal in nutritional value to the solutions that are normally used. To all erlenmeyer flasks 1 cc of an *Exobasidium japonicum* shake culture was added. Similar suspensions without fungicides as well as malt solutions without any addition were used as controls. After all cultures had been shaken for $2^1/_2$ to 3 weeks, the quantity of mycelium which had developed, was determined. The test cultures were filtered through a Buchner filter covered with filter paper. The latter with the mycelium was dried at a temperature of 105° C–110° C for 3 to 7 days, and then weighed. The experiments were replicated three times. It turned out that copperoxychloride suppressed the growth of the mycelium if used at the highest concentration (0.5 g/l). Lime sulphur also inhibited the growth of the mycelium, even in concentrations of 0.15 cc/l. Zineb did not seem to be of any influence; this fungicide even stimulated the growth. Though captan inhibited the growth to a high degree in concentrations of 0.2 g/l, this fungicide was of no influence at a concentration of 0.1 g/l. It was concluded that the effect of this fungicide and that of lime sulphur were most favourable (Fig. 8). Since the latter is now less widely used in practice, captan was chosen for the experiments with test plants.

10.4 Experiments with fungicides in vivo

Captan was used in concentrations of 2 g/l and 1 g/l. The buds of Azalea shoots were treated with the fungicide before, on the same day, and after the day on which they were inoculated with a pure culture of *Exobasidium*. In one series of experiments the tips of the shoots were removed simultaneously with the application of the fungal suspension. In a second series the tips were removed 10 days after the inoculation. It was inadvisable to remove the tips a long time before treating the plants with captan, as in that case the unfolding buds would develop their infections before the captan could have exercised its fungicidal action. In each series 4 plants were used with a total of 36 buds. From 4 control plants the tips of the shoots were

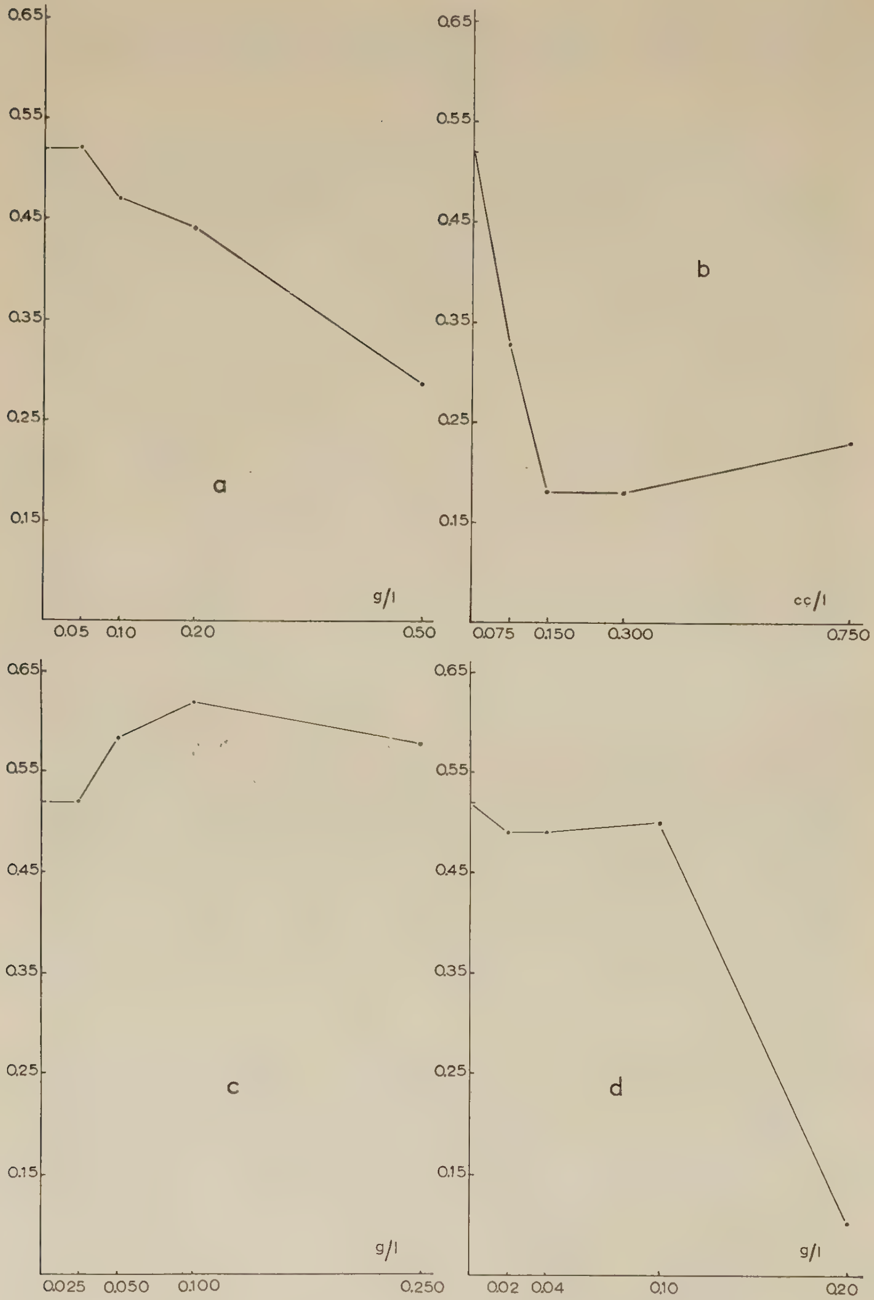


Fig. 8. Influence of fungicides on the dry-weight of mycelium formed by *Exobasidium japonicum* in shake cultures, *a*: copperoxychloride, *b*: lime sulphur, *c*: zineb, *d:* captan. Dry-weight of the mycelium without fungicides 0.52 g. Abscis: concentration in g or cc per l; ordinate: weight in g.

removed simultaneously with the inoculation, and from 4 other control plants this was done 10 days after the inoculation.

The smallest number of galls appeared when a captan suspension of 2 g/l was applied simultaneously with the fungal suspension (Table 9). The concentration of 2 g/l is the one that is also used in practice.

TABLE 9

Number of galls developing on groups of 4 Azalea plants with 36 buds in total, after inoculation with a suspension of *Exobasidium japonicum* and treatment with captan.

time of treatment with captan in relation to inoculation time	tips of the shoots removed simultaneously with inoculation		tips of the shoots removed 10 days after inoculation	
	concentration of captan		concentration of captan	
	1 g/l	2 g/l	1 g/l	2 g/l
2 days before	42	14	29	19
simultaneously	8	3	1	0
1 day later	20	15	8	6
7 days later	23	13	14	9

controls: 4 plants with the tips removed simultaneously with the inoculation: 34 galls
4 plants with the tips removed 10 days after inoculation: 24 galls.

From these experiments it became clear that, in case the buds remained dormant, the fungus was killed by captan when the latter was added simultaneously with the inoculum. If captan was administered one day or one week after the inoculum, galls became visible when the infected buds began to unfold. Perhaps the captan could not reach the sprouting blastospores because the latter were hidden between the scales of the buds, or because the fungus had already penetrated into the young leaves before the captan was applied.

In addition some minor experiments were performed with the other fungicides that had been tested in vitro. In these experiments the following solutions were used:

copperoxychloride 5 g/l
zineb 2.5 g/l
lime sulphur 7.5 cc/l

The test plants were sprayed one week after the inoculation, and the shoots were decapitated 10 days after that treatment. Each series was composed of 4 plants with in total about 30 axillary buds. The result is indicated in table 10.

The main series of experiments showed that fungicides are able to keep the fungus under control, provided that they are administered at the time of infection. This means that the buds should continuously be protected, as danger of infection is always present, particularly in those glasshouses where sporulating galls occur frequently.

Instead of spraying frequently, the application of hygienic measures seems to be more recommendable. Discarding the young galls before

TABLE 10

Number of galls developing on groups of 4 Azalea plants with 30 axillary buds in total after inoculation with a suspension of *Exobasidium japonicum* and treatment with fungicides.

fungicides	number of galls
copperoxychloride	3
zineb	8
lime sulphur	11
control plants	24

they sporulate, destroys the source of infection. This practice has also been recommended in the "Tuinbouwgids" (Dutch Horticultural Guide). However, it is difficult to pick out all young galls at the proper time. Therefore, in addition the grower may apply a fungicide to prevent infection, especially when sporulating galls are found. Spraying is also important when vegetative growth is resumed after pruning or after cuttings have been taken.

CHAPTER XI

DISCUSSION ON THE RELATIONS BETWEEN THE GENERA *EXOBASIDIUM*, *USTILAGO*, *TILLETIA* AND *TAPHRINA*

According to GÄUMANN (1949), the genera *Exobasidium* and *Kordyana* compose the family *Exobasidiaceae*. ALEXOPOULOS (1952) considers this family to be one of seven belonging to the order *Agaricales*, which according to others is synonymous with the order *Hymenomycetes* of the subclass *Holobasidiomycetes*, class *Basidiomycetes*. The *Exobasidiaceae* are primitive forms, lacking basidiocarps, and assignable to the *Agaricales* only on account of the presence of a hymenium consisting of basidia; this hymenium is developed just below the surface of the host plant and becomes exposed by the bursting of the cuticle. Because of this primitive character some authors, e.g. VIENNOT–BOURGIN (1949), assign the genera *Exobasidium* and *Kordyana* to a separate order: the *Exobasidiales*. Others, e.g. TALBOT (1954), are of opinion that the genus *Exobasidium* must be assigned to the subclass *Hemibasidiomycetes*, as there is a resemblance to the *Ustilaginales*. This resemblance is found in the fact that the *Exobasidiaceae* are parasites, and also in the budding of their basidiospores resulting in the formation of colonies; this reminds one of the budding of the sporidia arising from the smut spores of *Ustilago* species, such as *Ustilago hordei* (Pers.) Lagerh. Moreover, there is a striking resemblance between the characters shown by *Exobasidium* and by *Ustilago* species when grown in pure culture on an agar medium. Both fungi develop colonies with a wrinkled or folded surface.

In *Ustilago* species the process of budding is followed by conjugation of + and — sporidia. In the genus *Tilletia*, e.g. in *Tilletia caries* (D.C.) Tul., conjugation occurs between the sporidia as soon as they are formed. In this way in these two genera a dicaryotic

mycelium is formed. In the genus *Exobasidium*, however, conjugation has never been observed, though it is known that a dicaryotic mycelium is present in the host plant. This lack of conjugation is another point of difference between *Exobasidium* and the *Ustilaginales*. Though the genera *Exobasidium* and *Taphrina* are not closely related from a taxonomic point of view, the life cycles of both fungi show a striking ecological resemblance. This has already been pointed out by Woronin (1867), who mentions the following important similarities:

1. Both fungi are true parasites. They cause galls of a similar shape or, eventually, witches' brooms.
2. In both cases the mycelium lives in the intercellular spaces of the tissues. A layer of hyphae develops either between the epidermis and the cuticle of the galls or underneath the epidermis. The cuticle is burst at the time of sporulation.
3. In both cases germination of the spores occurs by budding.

Considering these similarities it is not astonishing that some authors consider both genera to belong to one group of which the members show the same kind of parasitism (Thomas, 1897; Juel, 1912). Laubert (1932) too mentions the analogy between the representatives of the genera *Taphrina* and *Exobasidium* in their way of living and in their influence on the host plants. In both genera fungi occur which cause galls and also witches' brooms. The galls show the same anatomical picture: a uniform, coarse parenchyma with few intercellular spaces and a scarcity of chloroplasts. In both cases the hyphae are thin, 0.5–1.0 μ in width, and grow in the intercellular spaces. The infection occurs by monocaryotic, haploid spores; the mycelium is dicaryotic. In *Exobasidium japonicum* no copulation of spores was found. In the genus *Taphrina*, according to Mix (1949), copulation seems to be exceptional. Only *Taphrina epiphylla* Sadeb. and *Taphrina deformans* (Berk.) Tul. show copulation of ascospores or of blastospores derived from the ascospores (Wieben, 1927; Mix, 1935). In the latter species, however, conjugation is rare. The difference between the genera exists in the presence of basidia in the genus *Exobasidium* and of asci in the genus *Taphrina*. The species of the former genus are mainly parasites on representatives of the *Ericaceae* in contrast to those of *Taphrina*, which do not occur on members of this family.

The method used by Graafland (1953) in cultivating *Exobasidium* species was originally used by Mix (1949) for growing *Taphrina* species. The cultures are in both cases alike, consisting of short hyphae, budding like yeast cells. The length of these short hyphae is not constant, but depends on the composition of the nutrient solution. Mix (1949) used the name "blastospores" for these yeast-like hyphae. He agrees with Fitzpatrick (1934) that these spores are infectious. Thus, the development, the multiplication by budding, the shape and the infectious character are similar in *Taphrina* and in *Exobasidium*. Mix's term "blastospores" can thus be taken over for *Exobasidium*. On the other hand Woronin (1867) called the sprouting cells "conidia". It seems, however, preferable to restrict this name to spores borne on conidiophores, as it is customary nowadays.

According to AINSWORTH and BISBY (1950), the term "sporidia" should be confined to the secundary spores formed by the *Ustilaginales* and *Uredinales*, where the process of budding is followed by conjugation of the sporidia.

Therefore, to avoid all confusion, in this paper the term "blastospores" has been used in accordance with the definition given by Ainsworth and Bisby: "a spore which has been budded off".

When comparing the parasitic character of representatives of the genera *Exobasidium* and *Taphrina*, it must be concluded that both genera are biologically very similar. This is remarkable, as the genera differ widely from a taxonomic point of view. In *Exobasidium* the spores are exogenous, borne on a basidium; in *Taphrina* they are endogenous, formed in asci. However, here the difference stops, for the behaviour of these spores is similar. By sprouting both may form blastospores, which may sprout once more. The basidiospores of *Exobasidium*, the ascospores of *Taphrina*, as well as the blastospores that are formed in both genera, are able to infect their host plants. One can imagine that the fungi belonging to these genera are of the same origin, and that, by differentiation, one group with endogenous and another group with exogenous spore formation have developed. However, it is also possible that the similarity in behaviour between these groups, so different in the taxonomically important mode of development of the spores, has been induced by their parasitic way of living. [1])

SUMMARY

1. The purpose of this investigation was to study the life cycle of *Exobasidium japonicum* Shir., a fungus parasitic on Azalea. The relation of this parasite to its host plant and the mode of infection were studied, as well as some control measures important to commercial growers. Attention was also paid to the question of the identity of this fungus with that described by Woronin as *Exobasidium vaccinii*, parasitic on *Vaccinium vitis idaea*.

2. For this study cultivars of the genus *Rhododendron* were chosen as host plants. Cultivars of Japanese origin viz. 'Esmeralda', 'Galestin' and 'Moederkensdag', frequently grown in Boskoop, and the cultivar 'Hexe', mainly grown in Ghent (Belgium), were used. Azaleas are cultivated during the greater part of the year in greenhouses at rather low temperatures, high humidity, and high moisture content of the soil. Water is frequently sprayed. The experiments were performed with rooted cuttings.

---

[1]) At the time the manuscript of this paper was ready to be sent to the printer a study of Göttgens appeared (GÖTTGENS, E. 1960. Untersuchungen über die Entwicklung von *Exobasidium Azaleae* Peck und seine Infektion der Wirtspflanzen unter besonderer Berücksichtigung der gallenbildenden Wirkstoffe. Phytopath. Z. **38**: 394–426). This author obtained pure cultures of the Azalea parasite in synthetic media and succeeded in infecting the host plant with basidiospores as well as with cell suspensions of pure cultures. Environmental conditions proved to be of influence on the length of the incubation period. Further Göttgens found that the culture filtrates contain activating substances, which promote the growth of tissue cultures of *Daucus carota* roots. As growth stimulating factors probably biotin, nicotinic acid or nicotinamide, inositol and pantothenic acid are present in the filtrate.

3. The first signs of attack by *Exobasidium japonicum* may appear even on the youngest just visible leaves of the unfolding buds. The diseased parts of the leaves are enlarged, and the blades become convex and crooked; these hypertrophic parts are called galls. In a young stage they are green, after ripening they become covered with spores, which give them a velvety appearance (Plate 1, A and B).

4. The dicaryotic, intercellular mycelium of the fungus forms basidia, which rupture the cuticle of the leaves. Each basidium bears four to five basidiospores (Fig. 1). The latter produce a great number of blastospores by budding. The tissue of the attacked parts of the leaves remains undifferentiated; both hypertrophy and hyperplasy of the mesophyll cells occurs (Fig. 2).

5. Colonies of *Exobasidium japonicum* were grown in pure cultures. As culture media malt and malt-saleb agar appeared to be most favourable. The fungus could also be grown in a malt solution in a shake culture. In both ways short hyphae and more or less regularly shaped, eventually fusiform cells, resembling blastospores were developed (Fig. 3).

6. The dormant buds were inoculated with basidiospores as well as with suspensions obtained from shake cultures. Simultaneously the shoots of the plants were decapitated in order to stimulate the development of the inoculated axillary buds. By this treatment the number of galls on the newly formed shoots appeared to increase considerably (Table 1). The source of the inoculum, whether basidiospores or cells from a shake culture, did not seem to influence the number of galls (Table 2).

7. The stage at which the fungus cells become dicaryotic was investigated. The basidiospores and the blastospores appeared to be uninucleate (Fig. 4 and 5). In vitro no cell fusion could be observed, neither between basidiospores nor between blastospores. Even between basidiospores and blastospores originating from one basidium, where in case of a sexual differentiation the presence of + and — spores could be expected, no fusion occurred (Fig. 6). The possibility, that cell fusion might take place on the surface of the leaf or after the fungus had penetrated into the leaf was also investigated. In that case at least one + and one — basidiospore, or a combination of their blastospores, would be necessary to accomplish infection. However, it appeared that blastospores originating either from a single cell out of a shake culture or from a single basidiospore were able to induce the development of galls (Table 3). Thus, it must be concluded that *Exobasidium japonicum* is homothallic.

8. By cross inoculations it appeared that *Exobasidium japonicum* did not attack *Vaccinium vitis idaea*, and that Azaleas were not attacked by *Exobasidium vaccinii* (Table 5). This difference in pathogenicity, added to a difference in colour of the colonies in vitro, are considered to be of specific importance, and for this reason *Exobasidium japonicum* Shir. is accepted as the valid name for the Azalea parasite. Strains of *Exobasidium japonicum* isolated from the Japanese Azaleas and from the cultivar 'Hexe', crossinoculated on the two host plants, proved to be identical in pathogenicity. (Table 4).

9. The leaves are probably susceptible to infection while still enclosed in the bud. By inoculating the leaves at different intervals after the buds began to unfold, i.e. after the tip of the shoot had been removed, it was shown that they remain susceptible until they have reached a length of 8–10 mm (Fig. 7, A-D; Plate 1, C-G and Table 6). In dormant axillary buds treated with a spore suspension, the fungus was still viable after 6 months, as after removal of the tip galls appeared on the unfolding leaves (Table 7).

10. The dormant buds should be protected by a fungicide against infection by wind-borne spores. Of the following fungicides different concentrations were assayed by adding them to a shake culture: copperoxychloride, lime sulphur, zineb and captan (Table 8). Captan strongly inhibited the growth of the mycelium at

a concentration of 0.2 g/l (Fig. 8). Buds on Azalea shoots were treated with this fungicide before, simultaneously with, and after inoculation with a spore suspension. The number of galls appeared to be most strongly reduced when the fungicide and the fungus inoculum were applied simultaneously (Table 9).

11. The similarity between the life cycle and the ecological behaviour of the genera *Exobasidium* and *Taphrina* is striking, though they are placed in quite different taxonomic groups, the *Basidiomycetes* and the *Ascomycetes* respectively. The relation of *Exobasidium* to the *Hemibasidiomycetes* is also discussed.

REFERENCES

Ainsworth, G. C. and G. R. Bisby. 1954. A dictionary of the fungi. Kew.

Alexopoulos, C. J. 1952. Introductory Mycology. New York, London.

Anonymus. 1956. Diseases of Azalea. Agric. Gaz. New South Wales **67**: 583–584.

Brefeld, O. 1889. Die Gattung *Exobasidium*. Unters. Gesammtgeb. Mykologie **8**: 12–18.

Burt, E. A. 1915. The *Thelephoraceae* of North America IV: *Exobasidium*. Ann. Missouri Bot. Gard. **2**: 627–658.

Ciferri, R. 1953. Cinque anni di esperienze con anticrittogamici a base di etilenbisditiocarbamato di zinco. Notiz. sulle Malat. delle Pianta **23**: (n.s. 2) 3–19.

Cole, J. R. 1944. Low-lime Bordeaux mixture controls leaf gall on Azaleas. Phytopathology **34**: 354–355.

Eftimiu, P. and S. Kharbush. 1927. Recherches histologiques sur les *Exobasidiées*. Rev. Path. Vég. et Ent. Agric. **14**: 62–88.

Ellis, J. B. 1874. New species of fungi found at New Field. N.J. Bull. Torrey Bot. Club **5**: 45–46.

Encke, F. 1960. *Rhododendron* in Pareys Blumengärtnerei 2. Aufl. **2**: 240–265.

Ezuka, A. 1955. Artificial culture of two species of *Exobasidium*: *Ex. vexans* Massee and *Ex. japonicum* Shirai. Bull. Tea Div. Tôkai–Kinki Agric. Exp. Stat. **3**: 28–53.

Farlow, W. G. 1878. List of fungi found in the vicinity of Boston. Bull. Bussey Inst. **2**: 247.

Fitzpatrick, R. E. 1934. The life history and parasitism of *Taphrina deformans*. Sc. Agric. **14**: 305–326.

Fuckel, L. 1861. Mykologisches. Botan. Zeit. **19**: 249.

Gadd, C. H. and C. A. Loos. 1948. The basidiospores of *Exobasidium vexans*. Trans. Brit. Myc. Soc. **31**: 229–233.

——— and ———. 1949. The fungus *Exobasidium vexans*. Tea Quart. **20**: 54–61.

——— and ———. 1950. Further observations on the spore-growth of *Exobasidium vexans*. Trans. Brit. Myc. Soc. **33**: 19–21.

Gäumann, E. A. 1949. Die Pilze. Basel.

Graafland, W. 1953. Four species of *Exobasidium* in pure culture. Acta Bot. Neerl. **1**: 516–522.

———. 1957. *Exobasidium japonicum* op *Azalea*. Med. Landb. Hogesch. en Opz. St. Gent **22**: 547–553.

Grootendorst, H. J. 1954. *Rhododendrons* en *Azaleas*, Boskoop.

Hilborn, M. T. and F. Hyland. 1956. The mode of infection of lowbush blueberry by *Exobasidium vaccinii*. Phytopathology **46**: 241.

Jaenichen, H. 1954. Krankheiten und Schädlinge in der *Azaleen*treiberei. Gartenw. **54**: 275–276.

Juel, H. O. 1912. Beiträge zur Kenntnis der Gattungen *Taphrina* und *Exobasidium*. Svensk Bot. Tidskrift **6**: 353–372.

Laubert, R. 1909. Über eine *Exobasidium*-Krankheit der Azaleen und den Mehltau des Apfelbaumes. Mitt. Kaiserl. Biol. Anst. Land- u. Forstwirtschaft **8**: 28–29.

———. 1925. Die "Klumpenblätter" — Krankheit der Azaleen und verwandte Pflanzenkrankheiten. Gartenw. **29**: 428–430.

———. 1932. *Exobasidiinae*. In P. Sorauer, Handbuch der Pflanzenkrankheiten **3**: 282–296.

LIND, J. 1913. *Exobasidiinae*. In E. Rostrup, Danish Fungi, Copenhagen, 350–352.
LOCKHART, C. L. 1958. Studies on red leaf disease of lowbush blueberries. Pl. Dis. Rep. **42**: 764–767.
LOOS, C. A. 1951. The causitive fungus. Tea Quart. **22**: 63–68.
MARCHAL, E. 1925. Eléments de Pathologie végétale, Paris.
MARCHIONATTO, J. B. 1929. Una micocecidia nueva para la Argentina. Bol. Min. Agric. Nac. **28**: 469–474.
MILES, H. W. 1928. Azalea culture in Belgium. J. Min. Agric. **35**: 137–147.
MIX, A. J. 1935. The life history of *Taphrina deformans*. Phytopathology **25**: 41–66.
———. 1949. A monograph of the genus *Taphrina*. Sc. Bull. Un. Kansas **33**: 1–167.
NAUMANN, A. 1909. Einiges über *Rhododendron*-Schädlinge. Jahresber. Ver. Ang. Botanik **7**: 171–188.
PECK, CH. H. 1874. Report of the botanist. Ann. Rep. New York State Museum of Nat. Hist. **26**: 72.
PETRI, L. 1907. Osservazioni sulle galle fogliari di *Azalea indica* prodotte dall' *Exobasidium discoideum* Ellis. Ann. Myc. **5**: 341–347.
RAALTE, D. VAN. 1955. Handboek der Bloemisterij. II. De belangrijkste cultures onder glas Doetichem.
RACIBORSKI, M. 1909. *Azalea pontica* im Sandomierer Walde und ihre Parasieten. Bull. Ac. Sc. Cracovie 385–391.
REITSMA, J. and J. H. VAN EMDEN. 1949. De bladpokkenziekte van de thee I. Bergcult. **18**: 218–231.
——— and ———. 1949. De bladpokkenziekte van de thee II. Bergcult. **18**: 370–377.
——— and ———. 1950. De bladgallenziekte van de thee in Indonesie. Arch. Theecult. **17**: 71–76.
RICHARDS, H. M. 1896. Notes on cultures of *Exobasidium andromedae* and of *Exobasidium vaccinii*. Bot. Gaz. **21**: 101–108.
RITZEMA BOS, J. 1901. Phytopathologisch Laboratorium "Willie Commelin Scholten". Verslag over de inlichtingen, gegeven in 1900. Landbouwk. Tijdschr. **9**: 65–125.
SAVILE, D. B. O. 1959. Notes on *Exobasidium*. Can. J. Bot. **37**: 641–656.
SHIRAI, M. 1896. Descriptions of some new japanese species of *Exobasidium*. Bot. Mag. Tokyo **10**: 51–54.
STAUTEMAS, E. 1951. De oorkensziekte bij *Azalea*. Tuinbouwber. **15**: 89–90.
TALBOT, P. H. B. 1954. Micromorphology of lower *Hymenomycetes*. Bothalia **6**: 249–299.
THIEL, J. 1951. Zwamziekten bij de *Azalea indica* L. Cult. en Hand. **17**: 494–496.
THOMAS, F. 1897. Über einige *Exobasidien* und *Exoasceen*. Forstl. Naturwiss. Zeitschr. **6**: 305–314.
TUBBS, F. R. 1947. A leaf disease of tea new to Ceylon. Tea Quart. **19**: 43–50.
VANDENDAEL, A. 1960. De export van Belgische sierteeltproducten. Belg. Tuinb. **41**: 157–161.
VIENNOT–BOURGIN, G. 1949. Les champignons parasites des plantes cultivées. Paris.
WELVAERT, W. 1952. De oortjesziekte. Rijksst. Plantenz. Gent. Leaflet 48.
WHITE, R. P. 1933. The insects and diseases of *Rhododendron* and *Azalea*. J. Econom. Entom. **26**: 631–640.
WIEBEN, M. 1927. Die Infektion, die Myzelüberwinterung, und die Kopulation bei *Exoasceen*. Forsch. Geb. Pfl. krankh. und Immunität im Pfl.reich **3**: 139–176.
WOLF, F. T. and F. A. WOLF. 1952. Pathology of *Camellia* leaves infected by *Exobasidium camelliae* var. *gracilis* Shirai. Phytopathology **42**: 147–149.
WORONIN, M. 1867. *Exobasidium vaccinii*. Ber. Verhandl. Naturforsch. Gesellsch. Freiburg **4**: 397–416.